comprising XRCC4 and DNA ligase IV.

The hamster c ll line XR-1 is hypersensitive to ionising radiation and displays defects in DNA double strand break repair. The XRCC4 locus has been shown to be deleted in XR-1 cells and the expression of the XRCC4 gene in these cells confers V(D)J recombination and partially restores double strand break repair (Li et al (1995) Cell 83: 1079-1083). The importance of XRCC4 in DNA double stranded break repair was therefore known prior to the present invention and this is confirmed by the Jackson declaration filed herewith.

The biological activity of XRCC4 is shown in the present specification to be intimately associated with DNA ligase IV. The two polypeptides are bound together under physiological conditions and neither exists in the cell in separation from the other. As evidenced by the Jackson declaration, this is strong evidence that DNA ligase IV is involved in the same pathways as XRCC4, *i.e.* DNA non-homologous end-joining.

It is alleged that the specification does not show the function of DNA ligase IV. However, a person of ordinary skill in art would reasonably conclude from the data presented in the specification that DNA ligase IV is part of the DNA non-homologous end-joining apparatus (see also figure 5). Evidence to support this is provided by the Jackson declaration and the various subsequent publications (Critchlow (1997) Current Biology 7 588-698, Grawunder (1997) Nature 388 (6641) 492-495, Grawunder et al (1998) Molecular Cell 2:477-484, Frank et al (1998) Nature 396:173-177, Riballo et al (1999) Current Biology 9:699-702). Furthermore, in the light of the above, a person of ordinary skill in the art would understand from the teaching of the present specification that the binding of DNA ligase IV and XRCC4 is important for DNA non-homologous end-joining and disruption of this binding would naturally have a deleterious effect on this activity.

Additional details of the mechanism of DNA non-homologous end-joining and the part played by each of the components of the DNA non-homologous end-joining are not needed in order for a skilled person to work the invention, in the same way that a car can be effectively immobilised by removing the rotor arm from its engine without any knowledge of what role the rotor arm actually plays within the engine. Further details of 'activity' or 'specific function' are simply unnecessary in order to teach the skilled person either what to do to work the invention or what will happen if he does.

The strong interaction between DNA ligase IV and XRCC4 is characterised experimentally in Example 1 of the present specification. Binding occurs between the C terminal region of DNA ligase IV and XRCC4 and this is disrupted by ionic detergents (e.g.

SDS) but not by high salt concentrations (e.g. 1 M NaCl). Indeed the interaction is strong and specific enough to allow the single st p purification of the expressed C terminal of DNA ligase IV from a bacterial lysate.

The present claims refer to the 'inhibition of binding' and rejections based on the term 'modulation' are therefore moot. 'Inhibition of binding' has a clear and definite meaning to the person of ordinary skill in the field. Detailed discussion of what is entailed by this term is found on page 13 line 29 to page 14 line 10 of the specification.

The Office Action alleges that no substantive guidance/direction regarding the assay method is given and absent exemplification of an a specific assay, the specification is not enabling for an assay method. As stated in the Jackson declaration, a person of ordinary skill is generally able to perform assay methods for compounds that inhibit binding between two proteins using routine skill and knowledge. In order to perform such an assay method, a skilled person requires only information about which proteins bind to each other and motivation that inhibiting such binding might produce beneficial effects. Both of these are provided by the present specification. There is therefore no question of undue experimentation being required to 'determine the format'. The assay method works with any experimental format and the skilled person simply has to 'plug in' the two proteins to whichever is most appropriate. Examples of assay formats, which are all well-known in the art, are provided on page 24 lines 6 to 22 and page 29 line 1 to page 32 line 19 of the application.

The Examiner alleges that the specification lacks exemplification of a specific assay, which is necessary in order to enable an 'assay method'. To be enabled, any person skilled in the art needs to be able to 'make and use' an assay method as claimed. As described above, on the basis of the specification, a skilled person is readily able to perform assay methods of the invention using routine methodology.

In addition to the routine methodology which is within the remit of the skilled person, the present specification provides experimental details of how an assay of the invention may be performed. Firstly, the skilled person is taught how to produce the polypeptides. XRCC4 is expressed and purified on page 71 lines 9 to 34 and DNA ligase IV is expressed and purified on page 73 line 32 to page 74 line 22. The skilled person is then taught how to immobilise XRCC4 on beads on page 74 lines 26-29 and how to bind ligase IV to the immobilised XRCC4 on page 74 lines 30-34. Finally, the skilled person is taught how to detect whether or not the ligase IV is bound on page 75 lines 1 to 11. Test compounds may be peptides as described on page 15 line 26 to page 19 line 7, antibodies as described on page 32 line 21 to page 35 line 32 or

chemical compounds as described on page 32 lines 16-19. All a skilled person needs to know to perform an assay method according to the invention is thus provided in the specification. Simply following the protocol provided and performing the binding step as taught in the presence of a test compound provides an assay method. A skilled person simply needs to be able to follow instructions. This does not amount to undue burden of experimentation. Thus, the specification teaches a skilled person all the technical steps for performing one example of a binding assay of the invention and assay methods are therefore fully enabled by the present specification.

The present claims are directed to assay methods for compounds that inhibit the binding of XRCC4 and DNA ligase IV. As described above, such methods follow directly from the discovery of a binding interaction between these two proteins. Assay methods could not be envisaged without knowing that two proteins specifically bind each other but are routine once this binding is known. Such methods thus reflect a practical application of the discovery of a binding interaction and are properly the subject of claims in the present application.

The specification is therefore enabling without undue experimentation for one skilled in the art to practice the present invention commensurate with the scope of the claims.

35 USC §112 SECOND PARAGRAPH

Claims 1-6, 19, 22 and 25 are rejected under 35 USC §112 second paragraph as failing to particularly point out and distinctly claim the subject-matter of the invention.

The present claims do not contain the terms 'substance', 'agent' or 'modulates' and recite proper markush language as indicated by the Examiner. Thus, rejections based on these points are therefore addressed.

Claims 3 and 6 indicate that a decrease in activity is indicative of the presence of an inhibitor, rendering moot objections thereto.

Claim 5 has been cancelled rendering moot objections thereto.

Claim 6 does not contain the term 'peptide fragments and variants' rendering moot objections thereto.

The terms 'includes' and 'including' have been removed from all the present claims, rendering moot objections thereto.

The present claims therefore particularly point out and distinctly claim the subject-matter of the invention and meet the requirements of 35 USC §112 second paragraph.

Conclusion

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number MEWE-005.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: January 22, 2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (amended) An assay method for [an agent] a compound which [modulates] inhibits the binding between XRCC4 (XR-1 Cell Complementing 4) and DNA ligase IV, or XRCC4 and DNA-PK_{CS}/Ku (DNA-dependent Protein Kinase catalytic subunit/Ku), or XRCC4, DNA ligase IV and DNA-PK_{CS}/Ku, the method comprising the steps of:

(i) bringing into contact [a substance which comprises] XRCC4, [or a peptide fragment of XRCC4 or a variant thereof which binds DNA ligase IV or DNA-PK_{CS}/Ku, a substance which comprises one or more components selected from the group consisting of DNA ligase IV or a peptide fragment of DNA ligase IV or a variant thereof which binds XRCC4 and DNA-PK_{CS}/Ku or a peptide fragment of DNA-PK_{CS}/Ku or a variant thereof which binds XRCC4] a test compound and one or more components selected from the group consisting of DNA ligase IV and DNA-PK_{CS}/Ku; [and a test compound] under conditions wherein, in the absence of said test compound being an inhibitor of binding [between said substances, said substances bind;] said XRCC4 binds to said one or more components selected from the group consisting of DNA ligase IV and DNA-PK_{CS}/Ku; and

(ii) determining binding between said XRCC4 and said one or more components selected from the group consisting of DNA ligase IV and DNA-PK_{CS}/Ku; [substances,]

reduction or abolition in binding between said [substances] XRCC4 and said one or more components selected from the group consisting of DNA ligase IV and DNA-PK_{CS}/Ku being indicative [of] that said test compound [being an agent which modulates] inhibits binding between XRCC4 and DNA ligase IV, or XRCC4 and DNA-PK_{CS}/Ku or XRCC4, DNA ligase IV and DNA-PK_{CS}/Ku.

2. (amended) An assay method for [an agent] a compound which [modulates] inhibits binding between XRCC4 and DNA ligase IV or XRCC4 and DNA-PK_{CS}/Ku, or XRCC4, DNA ligase IV and DNA-PK_{CS}/Ku, the method comprising the steps of:

(i) bringing into contact a [substance which comprises XRCC4 or a peptide fragment of XRCC4 which binds DNA ligase IV or DNA-PK_{CS}/Ku, or a variant thereof which binds DNA ligase IV or DNA-PK_{CS}/Ku, or which comprises DNA ligase IV or DNA-PK_{CS}/Ku or a peptide fragment of DNA ligase IV or DNA-PK_{CS}/Ku which [interacts with] binds XRCC4, or a variant thereof which binds XRCC4, and a] test compound and a polypeptide selected from the group consisting of XRCC4, DNA ligase IV and DNA-PK_{CS}/Ku; and

(ii) determining binding between said [substance] polypeptide and said test compound,

binding between said [substance] polypeptide and said test compound being indicative [of] that said test compound [being an agent which modulates] inhibits binding between XRCC4 and DNA ligase IV or XRCC4 and DNA-PK_{cs}/Ku or XRCC4, DNA ligase IV and DNA-PK_{cs}/Ku.

3. (amended) An assay method for [an agent] a compound which [modulates] inhibits DNA ligase IV activity, the method including the steps of:

(i) bringing into contact DNA ligase IV and a test compound; and

(ii) determining DNA ligase activity in the presence and the absence of test compound,

a difference in the activity in the presence relative to the absence of test compound being indicative [of] that said test compound [being an agent which modulates] inhibits the activity of DNA ligase IV.

Cancel claim 5 without prejudice.

6. (amended) An assay method [including] comprising

(i) bringing into contact a [substance which includes DNA-PKcs/Ku or a peptide fragment of DNA-PKcs/Ku or variant thereof which phosphorylates XRCC4, a substance which includes XRCC4 or a peptide fragment of XRCC4 or a variant thereof including a site phosphorylated by DNA-PKcs, and a] test compound, DNA-PKcs/Ku and XRCC4; and

(ii) determining phosphorylation [at said site] of said XRCC4 in the presence and absence of test compound;

a difference in phosphorylation in the presence relative to the absence of test compound being indicative [of] that said test compound [being an agent which modulates] inhibits the phosphorylation of XRCC4 by DNA-PK_{cs}/Ku.

19. (amended) A method comprising obtaining [an agent] a compound [able to modulate] which inhibits the binding between XRCC4 and DNA ligase IV, or XRCC4 and DNA-PK_{cs}/Ku, or XRCC4 and DNA ligase IV and DNA-PK_{cs}/Ku, employing a method according to claim 1 or claim 2; and, formulating said [agent] compound into a composition[including] which comprises a pharmaceutically acceptable excipient.

22. (amended) A method comprising obtaining [an agent] a compound which

[modulates] inhibits DNA ligase IV activity employing a method according to Claim 3 or claim 4 and formulating said [agent] compound into a composition [including] which comprises a pharmaceutically acceptable excipient.

25. (amended) A method comprising obtaining [an agent] a compound which [modulates] inhibits DNA-PKcs/Ku phosphorylation of XRCC4 employing a method according to claim 6 and formulating said [agent] compound into a composition [including] which comprises a pharmaceutically acceptable excipient.